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THE CYCLIC NATURE AND MAGNITUDE OF CELL DIVISION IN GASTRIC MUCOSA OF URODELE LARVAE REARED IN THE POND AND LABORATORY

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In a previous publication, Scheving, Chiakulas and Abzug (1959) reported that in salamander larvae the epidermal mitotic rate in animals from two different environments was characteristically cyclic over a 24-hour period. Although the cycle was manifest and corresponded as to time in both groups, the over-all mean mitotic rate of the epidermis in animals collected from the natural pond environment was 20 times that in the epidermis of laboratory-reared animals. A number of differences in the ecological conditions of the pond and laboratory environments were presented as possible factors responsible for the differential in the mitotic rates of the two groups. The results of our study on epidermis bring up a number of questions: (1) Do these ecological factors, possibly responsible for the differences in the mitotic rates, act specifically on the epidermis alone, since this tissue is directly exposed to the external environment, or do they act in an unspecific indirect manner so as to affect the general physiology of the animal and thereby affect the mitotic rate of other tissues as well? (2) Is the 24-hour cycle, characteristic of the mitotic rate of epidermis, present in other tissues?

To furnish possible answers to these questions, it was decided to study the magnitude and cyclic nature of the mitotic rate of the gastric mucosal epithelium of the same two groups of animals, pond and laboratory, used previously for the epidermal mitotic rate determinations. If a difference existed between the magnitude of mitosis in the two groups, a general physiological effect of environmental factors might be deduced. Other investigators (Klein and Geisel, 1947; Leblond and Stevens, 1948) have indicated that gut mucosal epithelium in the rat shows no significant cyclic variations in mitotic rate but that this tissue has a constant renewal rate. Therefore, it was of interest to determine if the phenomenon of daily cyclic variation in the mitotic rate of gastric mucosal epithelium existed in salamander larvae, and if so, what similarities or differences existed between the cycles of gastric mucosal epithelium and epidermis. The results of the determinations on the mitotic rate of gastric mucosal epithelium are the basis of this report.

Materials and Methods

Two groups of *Amblystoma punctatum* larvae, which were used in an earlier study of the epidermal mitotic rate (Scheving *et al.*, 1959), were used in the present study. The two groups were: (1) "laboratory" animals, which were reared in the laboratory from the egg stage until the chronological age of 6 weeks after hatching, and (2) "pond" animals, which were collected as larvae from the pond and which were approximately at the same stage of development as the laboratory animals when sacrificed. The differences in the two environments cannot be rigidly defined since no control procedures were used. It may be stated, however, that the laboratory animals were reared in city tap water which was run through commercial filters to remove the chlorine. In contrast, the pond animals grew in the semi-stagnant water of the natural pond. During the 24-hour collection period, water temperatures were taken hourly, and the temperature of the pond water ranged between 16° C. and 21° C. The laboratory animals were raised

Table I

Mean mitotic indices (mitoses/1000 cells) with standard deviations of gastric mucosal epithelium

Hour	Laboratory animals	Pond animals
6:30 A.M.	0.11 ± 0.07	2.89 ± 0.44
8:30 A.M.	0.33 ± 0.12	2.89 ± 0.69
10:30 A.M.	1.21 ± 0.40	5.65 ± 0.80
12:30 P.M.	1.09 ± 0.66	6.01 ± 1.62
2:30 P.M.	0.88 ± 0.35	4.60 ± 0.61
4:30 P.M.	0.97 ± 0.34	4.32 ± 1.11
6:30 P.M.	0.38 ± 0.40	4.14 ± 0.81
8:30 P.M.	0.31 ± 0.13	4.40 ± 0.85
10:30 P.M.	0.35 ± 1.14	4.60 ± 1.42
12:30 A.M.	0.41 ± 0.15	5.24 ± 0.92
2:30 A.M.	0.50 ± 0.12	7.02 ± 1.23
4:30 A.M.	0.43 ± 0.18	3.90 ± 0.63
Over-all Daily Mean	0.58 ± 0.34	4.64 ± 0.93

from the egg stage at heated room temperature, ranging from 20° C. to 24° C. During the 24-hour period of sacrifice, the room temperature averaged 21° C. Other possible differences between the two environments, such as differences in the oxygen tension and salt concentration of the water, degree of activity of the animals, were not determined.

Groups of 20 animals from each environment were sacrificed every two hours over a 24-hour period, fixed in Bouin's, sectioned transversely and stained with iron hematoxylin. Nuclear and mitotic counts were made on the gastric mucosal epithelium. Only the cells lining the lumen of the stomach were included in the counts; areas of continuity of the mucosal epithelium with the gastric glands were excluded. To avoid duplication, the number of nuclei and mitotic figures was recorded for every third section. At least 5000 nuclei were counted in each specimen. The mitotic index was calculated as the number of mitoses per 1000 cells. The mean mitotic indices for each time-period group and the mean over-all mitotic indices for the 24-hour periods, for both laboratory and pond animals, were determined and are indicated in Table I. Statistical comparisons and analyses were

made by computing the standard error of the differences between two means and the consequent probability.

Results

The mean mitotic indices of the gastric mucosal epithelium for each of the 12 time periods over a 24-hour day and for both animal groups are listed in Table I. When these indices are plotted in a graph (Figs. 1, 2), it is obvious that the mucosal mitotic activity of both the laboratory and pond animals is characteristically cyclic in nature.

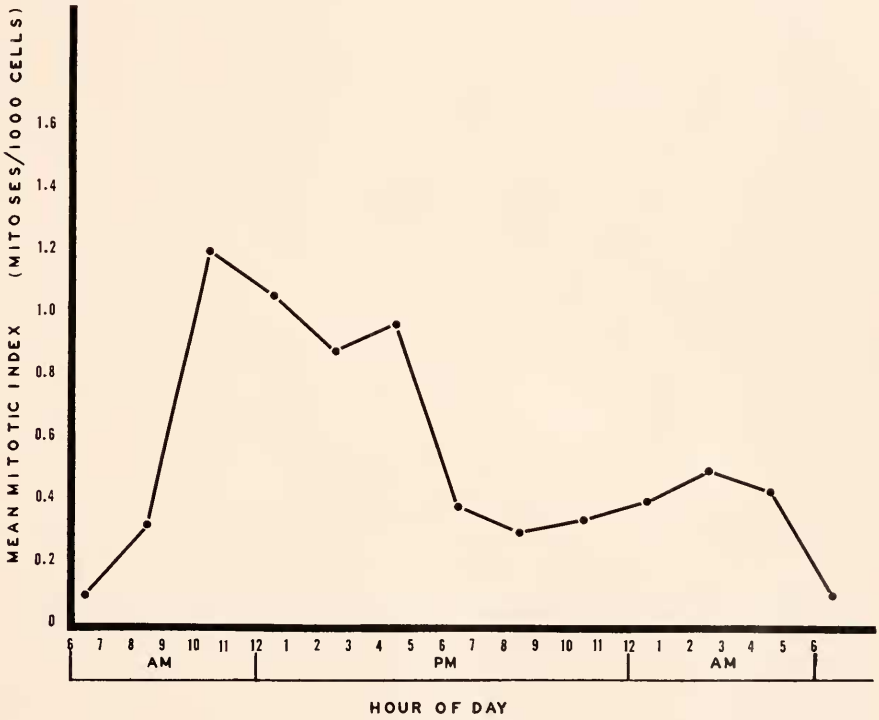

Figure 1. Mean indices, at two-hour intervals over a 24-hour period, for mitotic rate of gastric mucosal epithelium of laboratory animals.

In the laboratory animals the peak hour of activity is at 10:30 A.M., at which time the mean mitotic index is 1.21 ± 0.40 (Table I; Fig. 1). Following this peak, a gradual decrease in the number of dividing cells is recorded until 4:30 P.M., when the daily over-all mean mitotic index of 0.58 ± 0.34 is approached. Subsequently, there is a relatively rapid decrease in mitotic activity and the mitotic indices are below the 24-hour mean index during a seemingly stable period from 6:30 P.M. through 6:30 A.M., with the exception of a minor increase at 2:30 A.M.

The mitotic index of the 10:30 A.M. peak was compared with the over-all mean mitotic rate and the difference was found to be statistically significant ($0.001 < p < 0.01$). When the 10:30 A.M. peak index was compared to the 6:30 A.M.

minimum index, the difference indicated greater statistical significance ($p < 0.001$). It also can be seen from Table I and Figure 1 that the mitotic rate at 10:30 A.M. is twice the average rate for all hours and 10 times the minimum rate recorded at 6:30 A.M. The minor rise in the rate of cellular division observed at 2:30 A.M. has no statistical significance.

In the pond animals, two peaks of high cellular division occur—one at 12:30 P.M. when the mean mitotic index reaches the value of 6.01 ± 1.62, and another at 2:30 A.M. when the mean mitotic index of 7.02 ± 1.23 is recorded (Table I; Fig. 2). When these peak values are compared to the daily over-all mean mitotic

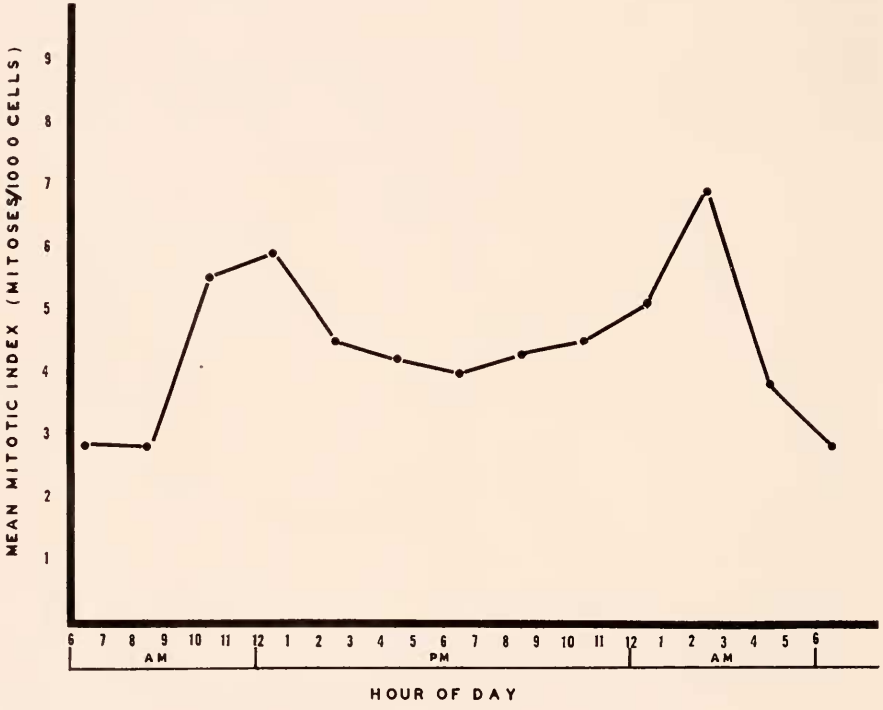

FIGURE 2. Mean indices, at two-hour intervals over a 24-hour period, for mitotic rate of gastric mucosal epithelium of pond animals.

index of 4.64 ± 0.93, they are not statistically significant. However, when these two values are compared to the minimum index of 2.89 ± 0.44 occurring at 6:30 A.M., both the differences are statistically significant ($p < 0.01$).

When the mitotic indices of both laboratory and pond animals are plotted on the same scale (Fig. 3) a number of interesting observations are made. It is evident from the graphs (Fig. 3) that the rate of cell division follows a similar cyclic pattern in both groups, with a high period of activity between 10:30 A.M. and 12:30 P.M. The 2:30 A.M. peak present in the pond group is not present in the laboratory group, although it is probable that the slight rise at 2:30 A.M. above the preceding period may represent a rather feeble acceleration corresponding to the peak present at this time in the pond group. Minimum mitotic activity

is established in both environmental groups between the hours of 6:30 A.M. and 8:30 A.M.

The most striking difference in the two animal groups is in the magnitude of the mitotic rates. The over-all mean mitotic rate in the gastric mucosa of the pond animals is approximately 8 times that of the laboratory animals (see Table 1; compare two graphs of Figure 3).

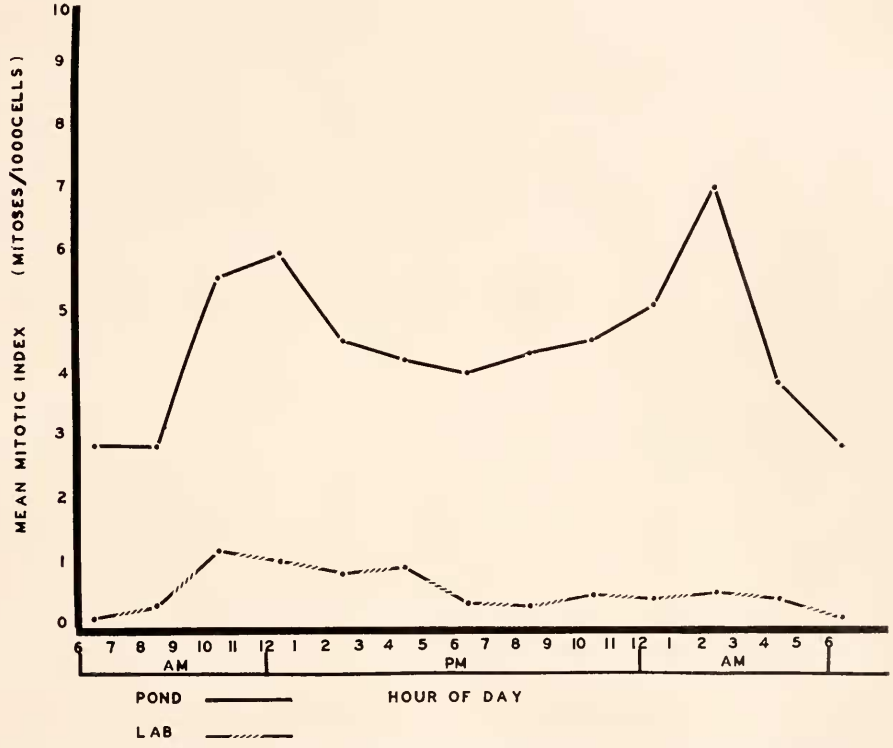

FIGURE 3. Mean indices of mucosal mitotic rate of laboratory and pond animals plotted on the same scale.

DISCUSSION

It is not the intent of this report to review the rapidly accumulating literature dealing with the cyclic nature of the rate of mitosis in various tissues or on the periodicity observed in many other biological processes. Brief summaries or reviews of some of the pertinent investigations in this field have been made by Scheving (1959), Leedale (1959) and Harker (1958). Here, we will discuss only the significance and implications of our results and relate and compare these results to those obtained in a similar investigation of the mitotic rate in urodele larval epidermis (Scheving *et al.*, 1959).

The results of this investigation, first of all, clearly demonstrate that the mitotic rate of the gastric mucosal epithelium is cyclic in character with occurrence of typical peaks and lows in the rate of cellular division during the 24-hour period.

Secondly, this finding indicates that gut mucosa is not a tissue with a constant replacement rate, at least in urodele larvae, and therefore this finding differs from the conclusions of Klein and Geisel (1947), and Leblond and Stevens (1948) that gut mucosal epithelium in the rat demonstrated no significant periodic fluctuations in its mitotic rate. Although a great number of previous investigations have dealt with periodicity of mitotic rate of epidermis or cornea, our results add to the evidence that other tissues may have similar cycles. What the basic factor underlying the cause of this cyclic nature of the cellular division rate is remains unanswered. However, whatever the cause, whether it be an intrinsic characteristic of the cells themselves or an externally imposed factor, it is apparent that it is operative in several tissues simultaneously.

When the graphs of Figure 3 are compared, it is noted that although the mucosal mitotic rate of both pond and laboratory animals is cyclic, the over-all daily mitotic rate of the pond group is 8 times that of the laboratory animals' rate. A similar but greater differential was reported for the epidermal mitotic rates of the same animals (Scheving *et al.*, 1959). Again, the reason for this difference cannot be explained at the present time. In the report on epidermis, a number of differences in the ecological factors of the pond and laboratory environments—such as oxygen tension, activity, diet, salt concentration—were mentioned as possible causes for the differences in mitotic rates between the two groups of animals. The implication was that these factors acted directly on the epidermis, since it was exposed to the external environment, or acted in some way to increase the general growth rate of the animals. Since this differential in mitotic rate is present also in the mucosal epithelium of the same animals, it appears that whatever the factors are that operate in producing this increased mitotic rate in pond over laboratory animals, they act in general to result in an enhanced rate of growth. However, this inference does not preclude the possibility that some of the ecological factors may still be acting directly on the epidermal tissue, because the gastric mucosal rate of the pond animals is only 8 times that of the laboratory animals, whereas the pond epidermal rate is 20 times that of the laboratory rate. Also, when it is considered that for the 24-hour period the mean mucosal mitotic index and the epidermal mitotic index of the laboratory animals are approximately the same, it is seen that the epidermis of the pond animals responds to a greater degree than does the mucosal epithelium. This is so probably because the epidermis is directly exposed to the environmental factors. In contrast, the mucosal epithelium, which is not directly exposed to the external environment, shows less of a mitotic response.

No correlation could be seen between the water temperature and the actual mitotic rate for specific hours. In the pond, the high temperature of 21° C. was recorded between 12:00 noon and 1:00 P.M. Correspondingly, a peak with an index of 6.01 ± 1.62 was seen in the mitotic cycle at 12:30 P.M. However, the highest peak in the cycle, with an index of 7.02 ± 1.23, occurred at 2:30 P.M. when the pond water temperature was 16.5° C. The cyclic nature of the mitotic rate thus appears to be unaffected by fluctuations in temperature over the 24-hour period. However, the mean mitotic rate over the 24 hours may be affected by the average temperature. The laboratory animals were raised at room temperature averaging 22° C. In contrast, the pond animals were subjected to colder temperatures in the pond during their period of growth, and on the day of collec-

tion the average pond temperature was only 18.5° C. Although pond and laboratory temperatures overlapped during some hours of the period over which the animals were sacrificed, at no time did the mitotic rates of the two groups show any overlap in magnitude.

The results of this investigation again point to the importance of knowing the 24-hour cyclic variations in the mitotic rates of tissues. When mitotic rates are used to interpret the results of experimental procedures, it is necessary to know if the animals involved were sacrificed at the same hours. Otherwise, comparisons and correlations or conclusions based on mitotic indices may be invalid. It is also of importance to know the conditions under which animals are reared, since differences in the environmental factors may cause great differences in the over-all mitotic rate of the tissues. Although in this investigation determinations were not made to establish the actual degree of difference between the pond and laboratory environments in terms of oxygen tension and salt concentration of the water, temperature, diet and animal activity, these differences may be responsible for the differentials seen between the mitotic rates of the two groups of animals. Controlled experiments are in progress to test the effect of each individual factor on mitotic rate of tissues.

Summary

1. The mitotic rate of gastric mucosal epithelium in both pond- and laboratory-reared urodele larvae is cyclic over a 24-hour period. Maximum rate of cell division occurs in both groups between the hours of 10:30 A.M. and 12:30 P.M.; minimum mitotic activity is recorded between 6:30 A.M. and 8:30 A.M.

2. In pond animals, the mitotic rate of gastric mucosa is 8 times the rate of the mucosa of laboratory-reared animals. The environmental differences between pond and laboratory are suggested as the underlying cause for the differential in the rates of mitosis in the two groups of animals. Since a similar difference was previously found in the epidermal mitotic rates of the same animals, it is suggested that in the pond environment an enhanced rate of growth is present.

3. The difference between the pond and laboratory mucosal daily mean mitotic rate is of lesser magnitude than the difference between the epidermal rates of the same animals. This fact indicates that the epidermis may be responding directly to exogenous environmental factors, whereas the mucosa responds only to a lesser degree since it is not directly exposed to the environmental factors.

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